ANOPHELES INFECTIVITY EXPERIMENTS.

AN ATTEMPT TO DETERMINE THE NUMBER OF PERSONS ONE MOSQUITO CAN INFECT WITH MALARIA.

By M. BRUIN MITZMAIN, Technical Assistant, United States Public Health Service.

The habits of a blood-sucking insect often give the investigator a clue to its relations in the part played by it in the economy of man. A mosquito which, ticklike, satisfies its food demands by a prolonged, uninterrupted aspiration of blood and resumes its normal function as regards procreation without a second blood meal, does not play a rôle which need attract the attention of the sanitarian. Opposed to this form, however, a mosquito like the plasmodia-bearing *Anopheles* shows its evil possibilities at the outset by its insatiable food requirements. It may demand three complete meals of blood before providing for one of several egg layings. It may be in the habit of taking a short meal and, interrupting itself automatically, leave one host in order to rest or to attack another host. Although it has been found that an anopheline mosquito may survive for a period of 67 days without partaking of blood, a typical member of the genus requires a blood meal approximately every three days in order to thrive and perpetuate its kind. When a full meal is not taken, due to an interruption on the part of mosquito or host, it is necessary to consummate its purpose in a further attack on the same or a different host. In this way the anopheline mosquito becomes an important factor from the point of view of disease transmission. Bearing this in mind, the sanitarian must then determine to what extent a mosquito which has already contaminated its gustatory parts in biting a malaria-diseased human being becomes a menace to the public health.

The whole question of the importance of the mosquito as a vector of malaria resolves itself into a consideration of numerical potentialities. How many mosquitoes succeed in obtaining a blood meal, and of these what percentage succeeds in developing the malarial cycle? Ronald Ross estimates that only 1 out of 24 anophelines manages to bite a human being, and this proportion is greatly reduced, economically measured, because less than 25 per cent succeed in the rôle of carriers. Roughly considered, then, we may look upon 1 out of every 100 female anophelines in a malarious country as a possible infecting agent. This one mosquito, regarded as a health disturber, must be evaluated in terms of certain biologic factors in order to appreciate the potency and extent of its parasitism in the conveyance of malaria.

As a result of feeding tests the writer desires to emphasize the individuality of the insect carrier. The virility of one or a few individuals in the relationship to malarial conveyance is here demonstrated and leads one to infer that the power of an infected *Anopheles*

to harbor the fever organisms probably remains until the time when it is no longer able to bite. This length of time, the longevity of infection, is considerable and probably has not been fully determined. In the present experiments the maximum has been found to be a period of 25 days.

If it can be established that a malarial mosquito once infected remains so, it would appear an added contribution to our knowledge to at least approximate the extent of this infectivity. Is every bite an infective one, and how many persons in a community can be infected by the same mosquito? A partial solution of these problems is attempted in this series of experiments. Here 14 cases of malarial fever resulted from the bites of infected mosquitoes. In 11 of these successful transmission resulted from mosquito biting which was purposely interrupted in order to induce the insects to bite as many persons as was practicable. Applying the mosquitoes so that one person after another was bitten in rapid succession (or at short intervals), it was indicated that in 9 of these experiments successful conveyance of malaria resulted.

It has been demonstrated heretofore, and confirmed by the writer, that the bite of a single *Anopheles* can cause malarial fever, and it would appear of sanitary significance to determine if an *Anopheles* can convey infection repeatedly without again obtaining blood from the original source of infection. This successive infectibility would have a practical bearing in community sanitation, indicating the necessity for intensive elimination of mosquitoes. In the case of an *Anopheles* which does not travel far, preferring to rest and obtain its food in buildings frequented by people, the importance of local infectibility is obvious.

Anopheles punctipennis Say, the species here considered, though not found commonly in residences, is of frequent occurrence in locations equally favorable for infection, such as privies. Epidemiologically, school privies and similar places offer potentialities in this regard which the sanitarian can not afford to underestimate.

Castellani and Chalmers[1] (1913) consider the infection of several persons by a single mosquito as probable. They state: "The anopheline not merely carries the germ but because of its length of life a single individual may be capable of infecting several human beings; for it must be remembered that there is no proof that the mosquito is in any way deleteriously affected by the malarial parasite."

Stephens[2] (1911) considers the question of how many persons one anopheline can infect as one of the problems in the infection of mos-

[1] Castellani and Chalmers (1913), Manual of Tropical Medicine, second edition. Wm. Wood & Co., New York.

[2] Stephens, J. W. W. (May, 1911), Methods for detecting sporozoites and zygotes in mosquitoes infected with malaria. Bulletin of Entomological Research, vol. 2, p. 7.

quitoes which need further investigation. He states that we have no data on this point.

In discussing the mode by which sporozoites are introduced into the blood of man and the subsequent fate of the malarial parasite, Braun[1] (1908) writes:

> If a small number only happen to be present in the salivary glands of the mosquito, they are all discharged with its first bite; in other cases only a few are gotten rid of, so that under these circumstances one single *Anopheles* is capable of infecting several persons.

Ross[2] (1910), discussing the proportion of mosquitoes which succeed in biting human beings, states that in the huts of poor natives, and the badly managed barracks and hospitals where many unprotected people sleep in the same room, a single mosquito may often be able to bite several persons during one night. In such houses the chances of infection must be enormously increased, and the practice of congregate sleeping must be one of the principal causes of the diffusion of malaria.

The work here described was conducted in the course of malarial investigations under the charge of Surg. R. H. von Ezdorf, United States Public Health Service, and the findings and the care of the cases had his supervision and confirmation.

In an effort to ascertain the infectibility of *Anopheles punctipennis*, several volunteers were bitten successively by a series of three mosquitoes, which had been applied 10 days or more previously to an uncomplicated case of benign tertian malaria.

An untreated patient suffering from tertian malarial fever volunteered for the purpose of furnishing the parasites required in this experiment. During the interval of awaiting gametocyte development (January 17 to February 6, 1916), several generations of complete schizogony were observed to occur in the patient's blood. However, sporogony was unduly inhibited up to January 29, too few gametocytes being present to warrant infectivity of the insect hosts. Finally, sufficient numbers of sexual forms matured during the period from January 29 to February 6, at the termination of which period (February 6 and 7) mosquitoes were applied to the patient.

The paucity of sexual parasites in the blood of the donor may be appreciated from the counts made in a thick film and a thin film prepared February 7. In the two preparations 1,231 leucocytes were counted and the matured gametocytes encountered numbered two, an average of one gametocyte to 616 leucocytes. In addition to these there were observed, in the two blood specimens, 48 half-grown gametocytes and five ranging in size from three-fourths to nearly full

[1] Braun, Max (1908), The Animal Parasites of Man, third edition, p. 100. Wood & Co., New York.
[2] Ronald Ross (1910), The Prevention of Malaria, p. 169. John Murray, London.

grown forms. At the time of these examinations the patient had been started on a course of quinine treatment.

It was planned to have only one of the experimental mosquitoes bite several persons, but unfortunately it appeared that not any one of the four specimens available was vigorous enough to survive the full length of the experiment. On this account it was feared that biting the first volunteer and waiting the usual two weeks of incubation would not provide for sufficient longevity to complete the series of successive feedings. In applying the mosquitoes it was aimed to give a practical bearing to the investigation by both the "complete" and "interrupted" methods of feeding, in order to find if it were possible that the same mosquitoes could infect within the same day and during an interval of a few hours at most. This has its significance in the infecting of a group of people either assembled or as individuals visiting the same public places at short intervals.

Among the persons who volunteered for the experimental inoculations are included five physicians to whom the writer is indebted also for their clinical histories. The general interest and personal sacrifice of these persons made feasible the scope of this investigation.

It was aimed at first to apply the four specimens in parallel series to as many persons as would volunteer, but it was found that the mosquitoes were too feeble to warrant this division. Therefore as many of the four as could be induced to bite were used for each person after the preliminary feeding. On February 17 one of the specimens died (No. 18), and not more than two of the remaining specimens could be induced to bite the different volunteers. In some instances the mosquitoes when applied were interrupted in their biting after a few seconds; in other instances they were given opportunity to feed to repletion.

Control of persons volunteering.—Although ideal conditions governing control of the persons employed in the experiments were not secured, the following circumstances indicate practical elimination from accidental or external infections. All persons involved were examined previously—the blood smears in all cases were negative for parasites. The experiments were conducted out of the malarial season for this locality. During this period there was not an instance of a report of clinical symptoms in any person on the hospital reservation not employed for these tests.

The following table enumerates the persons bitten, the length of time mosquitoes were applied, and the resulting incubation period in the positive cases:

FIG. 1.—SPOROZOITES FROM A CRUSHED LOBE OF THE SALIVARY GLANDS OF MOSQUITO NO. 23 AFTER IT HAD BITTEN 12 PERSONS.

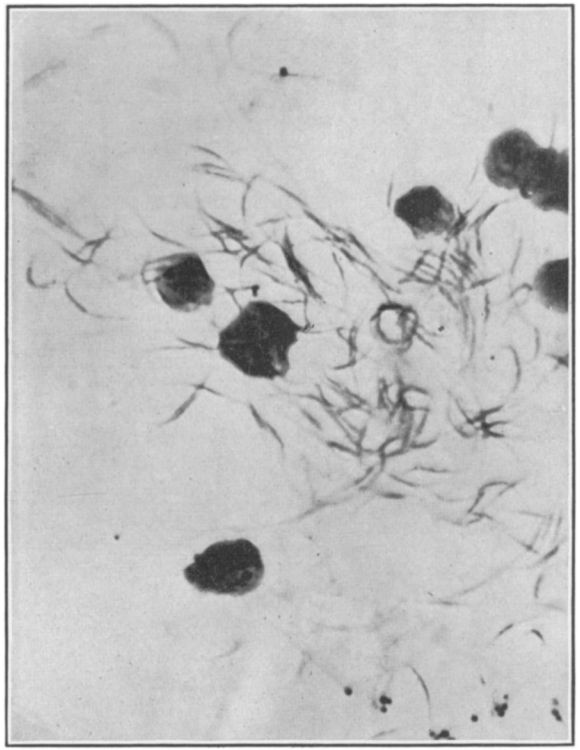

FIG. 2.—SAME AS FIG. 1, ENLARGED.

APPEARANCE OF THE STAINED SPOROZOITES WITHIN THE SALIVARY GLANDS OF MOSQUITO NO. 24.

Length of time each person was bitten and incubation period resulting.

Date bitten.	Volunteer.	Infected A. punctipennis applied.				Incubation period (days).
		No. 18.	No. 23.	No. 24.	No. 25.	
1916.						
Feb. 17	H. E. H.	1 minute	45 seconds	45 seconds	40 seconds	15
18	Dr. H. A. T.		Complete	Complete		14
21	Dr. R. C. D.			40 seconds	1 minute	13
21	Dr. T. H. D. G.			30 seconds	40 seconds	14
21	F. M. H.			1 minute	45 seconds	14
21	R. E. T.			30 seconds	80 seconds	15
22	Dr. R. T. O'N.			1 minute		15
23	H. T.		40 seconds	1 minute		13
23	Dr. S. L. C.		40 seconds	30 seconds		13
26	F. W.		50 seconds			16
26	F. A.		1 minute	1 minute		14
28	C. B.		Complete	Complete		13
Mar. 1	T. A. R.		35 seconds			19
1	G. O.		1 minute			18
2	J. M.		50 seconds			Neg.
2	J. W.		1 minute			Neg.
3	W. P.		20 seconds			Neg.

In every instance in which two mosquitoes succeeded in transmitting infection, at least one of the pair was proved to be capable of causing the disease when used singly, namely, Nos. 24 and 25 in four cases; Nos. 23 and 24 in five cases.

No. 24 was proved to be the sole infective agent in one experiment. No. 23 was proved to be the sole infective agent in three experiments.

The degree of parasitism in the four mosquitoes used in the inoculations is described in a previous report and is given as follows:

No. 18.—Dissected February 19. Incubation 12 days. The structures of the midgut were fairly obscured by the projecting mass of oocysts. Twenty-eight of these bodies were counted; seven were observed to contain sporoblasts, while the majority of the remainder contained varying numbers of spindle organisms in different stages of development. Several of these bodies ruptured upon slight pressure, when swarms of relatively small sporozoites floated away from the ripe oocysts. The salivary glands in this specimen were heavily parasitized. Five of the lobes appeared granular with matted clusters of typical curved organisms. Numerous sporozoites were seen moving sluggishly through the saline medium.

No. 23.—Dissected March 3. Incubation period 25 days. This specimen had been given a feeding of human blood five hours previously, consequently the alimentary tract was found engorged with blood. No oocysts or shrunken capsules were seen. Several sporozoites were seen moving freely in the solution surrounding the dissected stomach. The salivary glands were greatly hypertrophied; those lobes which in the dissection were torn loose with the head were parasitized with a moderate number of sporozoites, and a remaining single lobe afterwards removed from the thoracic cavity was seen to be much shrunken in size, its degenerated cells containing only a few sporozoites. A large number of these organisms were observed free and very active in some material dissected from the thorax.

No. 24.—Dissected March 1. Incubation period 24 days. The midgut was seen with four oocystic capsules, three much shrunken, narrowed to probably one-half the width. One appeared normal in size, only slightly shrunken, containing three nonmotile sporozoite-like bodies. The dissected salivary glands were not removed cleanly either from the head or thorax. A small portion was attached to the head and two

lobes of the glands were seen in material crushed from the thorax. On pressure, numerous very active sporozoites were observed. The gland structure was granular, but no organisms were perceived in the cells on account of the presence of the mat of overlying fungous threads.

No. 25.—Dissected February 23. Incubation period, 16 days. The stomach of this specimen was not seen on account of the superimposed tangle of mycelium threads of some fungous parasite which probably caused the insect's death. When the gut wall was pressed a few sluggishly motile sporozoites were observed. These originated probably from a gut oocyst. Only small disjointed portions of the salivary glands were examined. These appeared heavily parasitized with more or less active sporozoites possessing the typical large refractile nucleus and sharply curved ends.

The following protocol accounts for the results of mosquito biting in the various volunteers, presenting the clinical histories and the degree of parasitism involved.

H. E. H.—Previous history of malaria: Negative.

February 17, 1916. Bitten by mosquitoes Nos. 18, 23, 24, and 25.

March 2. First distinct clinical symptoms of malaria observed in H. E. H. Severe headache for past three days. Slight chill with pain in joints and chest. No parasites seen. Temperature, p. m. 39° C.

March 3. Temperature, a. m. 37.4° C.; slight chills with increasing temperature to 38.8° C. Pains general, vertigo and lassitude. Parasites in finger prick blood: A few half grown rings; several ameboid forms and one exhibiting Schuffners granules; organisms distinctively *P. vivax.* Given 30 grains quinine bisulphate.

March 4. During night, fever and sweat. Temperature normal in the morning, showing effects of having taken 40 grains quinine bisulphate.

Dr. H. A. T.—Previous history of malaria: Suffered with tertian malaria in 1912. Recovered by quinine medication.

February 18, 1916. Bitten by mosquitoes Nos. 23 and 24.

March 2. Complains of severe headache and eye strain; general lassitude. Temperature normal.

March 4. Premonitory symptoms all morning. Parasites seen prior to chill. Scarce and very small rings in blood. One large distinct *vivax* and several small ameboid rings seen. Severe chills at 4.30 p. m.; previously, temperature at 10 a. m., 37.6°, rising to 38.6°, then 39° just one hour previous to chill.

March 4. Temperature during last night rose to 40° C.; febrile paroxysm severe. Morning temperature, 37.8° C. Parasites few; large ameboid parasites—very characteristic pure tertian.

March 5. Temperature, 39.° C. Quinine taken.

Dr. R. C. D.—Tertian malaria in 1907; recovered.

February 21, 1916. Bitten by mosquitoes No. 24 and 25; pronounced local reaction at site of each bite.

March 2. Languor, slight headache, and muscular pains. Blood examination negative.

March 3. Headache and malaise. Blood examination negative.

March 4. Chilly sensation beginning at noon; severe chill and headache at 7 p. m.

March 5. Chilly sensation at noon, severe headache and muscular aching. Young ring forms of parasites found in smears. Commenced quinine treatment.

March 6. Chill at 11 a. m. Few large rings of *P. vivax.*

March 7. Headache and muscular soreness throughout day.

March 8. No notable symptoms.

March 9. No notable symptoms.

Dr. T. H. D. G.—Previous history of malaria: Estivo-autumnal attacks in September, 1915, cured with quinine.

February 21, 1916. Bitten by mosquitoes Nos. 24 and 25.

March 4 to 6. Nervousness without other symptoms.

March 6, a. m. Slight headache and aching joints, ringing of ears; general lassitude. Temperature, 8.15 p. m., 38.9° C. Acute joint pains, headache, slight dizziness, feet burning. Blood examination at 9 p. m. (thin smear); observed three very distinct large young rings. Quinine begun 10 p. m.

May 19. Relapse. Clinical symptoms and *P. vivax* in blood. Only pure tertian forms found; also gametocytes on blood examination.

F. H.—Previous history of malaria: Latter part of August, 1915, had malarial fever in New Orleans, La. Treatment given at Government hospital, 20 grains quinine bisulphate daily, alternating with 40 grains daily for two weeks, then 10 grains daily for six weeks. Total administered, 840 grains of quinine bisulphate in acid solution. Blood examination in October, 1915, showed absence of parasites, and none upon subsequent examinations.

Feb. 21, 1916. Bitten by mosquitoes Nos. 24 and 25.

March 7. Headache and general lassitude. Moderate number of half-grown schizonts (*P. vivax*) found.

March 9. A moderate number of well-formed pigmented schizonts, in addition to some large characteristic *vivax* rings seen.

March 10. Distinct chill with numerous asexual parasites at all stages in blood. Specific treatment begun.

R. E. T.—Previous history of malaria: Negative.

February 21, 1916. Bitten by mosquitoes Nos. 24 and 25.

March 4. Symptoms commence. Headache and aching legs. Feeling generally slightly "off" for two days, March 4 to 6.

March 6, 2.30 p. m., temperature, 38.4° C.; 4 p. m., temperature 39.4° C.; 7 p. m., 38.8° C. Retired late afternoon with slight chill. Temperature at this time 37.8° C. Treated with quinine.

March 7. A few characteristic parasites of tertian malaria found.

March 8. *P. vivax* represented by moderate number of segmenting schizonts.

Dr. R. T. O'N.—Previous history of malaria: Estivo-autumnal in 1913; cured with quinine.

February 22, 1916. Bitten by mosquito No. 24.

March 3. Complained of pain in back and neck.

March 4. Same symptoms aggravated.

March 5. Dizziness and nausea. Experienced slight chill, followed by rise of temperature and sweating; confined to bed.

March 8. No parasites seen until today. There were present a few large forms and presegmenting pigmented schizonts. Quinine treatment begun.

H. T.—Previous history of malaria: No symptoms since August, 1914.

February 23, 1916. Bitten by mosquitoes Nos. 23 and 24.

March 6. Complained this afternoon of burning sensation of ears and back of head and neck. Experienced chill at night followed by fever of 39.0° C.

March 7. Generally weak and sweating profusely. Morning temperature of 38.0° C, gradually falling to 37.4° C. Confined to bed. Specific treatment begun. Blood examination showed a small number of tertian rings in size up to half-grown ameboid forms with pigment.

May 1. Relapse. Chill and fever probably malarial. Quinine taken indifferently.

May 22. Moderate numbers of *P. vivax* in all forms present in blood.

Dr. S. L. C.—Previous history of malaria: None since July, 1911—recovered from tertian attack.

February 23, 1916. Bitten by mosquitoes Nos. 23 and 24.

March 4 and 5. Severe headache, eyes congested, and chest sore.

March 6, afternoon. Aching joints, slight fever, gradually reaching 38.8° C. At this time blood examination proved negative.

March 7. A scanty number of typical tertian schizonts of the ameboid type found in two thin smears, and a few young rings seen in a thick smear. Specific treatment begun in the evening.

F. W.—Previous history of malaria negative.

February 26, 1916. Bitten by mosquito No. 23.

March 13. No symptoms with the exception of a slight headache. Parasites present; a very few trophozoites seen.

March 14. Headache and chilly sensations, knees stiff. Temperature 38.9° C. at noon falling to normal.

March 15. Slight chill at night, temperature 38.0° C. Complains of pain in knee joints and stiff neck. Scanty numbers of typical half-grown parasites in blood. Quinine given.

F. A.—Previous history of malaria: Had malarial attack in October, 1912, cured by specific treatment.

February 26, 1916. Bitten by mosquitoes Nos. 23 and 24.

March 11. Experienced prodromal symptoms beginning in the morning with headache; backache and aching joints in the evening. Blood examination made in the morning was negative except for two doubtful rings. Later in the evening a few distinct rings of *P. vivax* found in both thick and thin smears.

March 12. Symptoms more acute; aching in back, head, neck, and joints; temperature normal.

March 13. Complains of severe headache and pain in back.

March 14. Symptoms remain same. A moderate number of large tertian rings seen in blood. Given quinine this date.

C. B.—Had malaria in December, 1913, from which he recovered when given specific treatment.

February 28, 1916. Bitten by mosquitoes Nos. 23 and 24.

March 12, 11 a. m. Found a moderate number of merozoitlike parasites in blood serum and on edge of blood cells; and in one instance a parasite was found contiguous to a red cell. Gave no sign of illness of any sort at the time of blood examination.

March 13. Complained of feeling cold and was perspiring afterwards. Slight frontal headache at this time. Responded readily to quinine treatment.

T. A. R.—Previous history of malaria negative.

March 1, 1916. Bitten by mosquito No. 23.

March 19. Complained of headache, pain in back, and joints.

March 20. Pains continued in severity. Temperature 38.2° C. at 7 a. m.; chill at 9.30 a. m., lasting 10 to 15 minutes, followed by temperature 39.6° C. and vomiting. Temperature 38.6° C. at 6.30 p. m.

March 21. Small ring parasites found. Had distinct chill and vomiting. Moderate number of large ameboid parasites, several with pigment (*P. vivax*). Given specific treatment from this date.

G. O.—Previous history of malaria negative.

March 1, 1916. Bitten by mosquito No. 23.

March 19. Headache, pain in joints, general weakness. Temperature 38.6° C. at 4.15 p. m. Distinct chill at 6.30 p. m., followed by temperature 39.2° C. At 10 p. m. temperature 38.4° C., perspiring freely.

March 20. General weakness, pain in stomach, vomiting—temperature normal.

March 21. A moderate number of distinct small *vivax* rings seen in blood smears. Several ameboid forms with pigment noted. Quinine treatment given.

It is probably of little more than academic interest to ascertain whether the infection of the human host is derived from the biting in the preliminary stage or at the completion of the bite. Several authors have suggested the greater probability of the former method, among these Ross, who says, "I think that mosquitoes inject their poison before commencing to suck."

Smith[1] (1912), regarding the salivary excretion as a poison fluid in its action on the infected host states, "The pain is caused entirely by the action of the poi on in breaking up the blood, and as the first act of a biting mosquito is to introduce this poison into the wound, the pain and inflammation will be the same whether the insect gets its meal or not." He states further in agreement with the hypothesis of Reamur that the poisonous saliva, introduced by the parasite, functions as an anticoagulin acting just upon so much blood imbibed.

An attempt was made to ascertain if infection would follow a very short exposure to the bite of infected mosquitoes. In several instances in applying the specimens by the interrupted method, several persons were bitten in succession, and the mosquito was permitted only to insert its proboscis for a few seconds, then interrupted. For example, in one day's feeding with mosquitoes Nos. 24 and 25 four persons were bitten in the course of 2 hours and 30 minutes. The mosquitoes were of course interrupted in all but the last feeding, so that they did not become engorged upon the blood of any one person, but from the group of persons.

In every instance ample evidence was obtained to show that biting took place to the extent that distinct macules were seen on the arm of the persons serving as hosts, and the mouth parts and other visible structures of the mosquito involved were carefully examined during the biting process with a strong hand lens. Mosquitoes Nos. 24 and 25 were permitted to apply their rostrums to the fullest extent, well inserted, for periods ranging from 20 seconds to 1 minute and 20 seconds; Dr. R. C. D. was bitten at 2.30 p. m., followed by Dr. T. H. D. G. at 4 p. m., F. M. H. at 4.35 p. m., and R. E. T. at 4.55 p. m.

It is evident that for infection a short bite was quite sufficient for conveying the parasites.

In the last two experiments outlined in Table No. I, in which successful inoculations were effected (T. A. R. and G. O.), both persons were bitten by mosquito No. 23 within an interval of five minutes. Here the interruption in both inoculations was abrupt; the mosquito was not given an opportunity to complete its meal upon either host. The subsequent incubation periods were protracted, and the *Plasmodium* was not recovered before the 18th–

[1] Smith quoted by Howard, Dyar, and Knab (1912). The mosquitoes of North and Central America and the West Indies. Vol. I, pp. 317-318.

19th day in these instances. The three applications of this same mosquito during the following two days resulted negatively. It was observed that the biting was extremely feeble and none of the hosts felt the pain of the biting indicative of successful inoculation. This was demonstrated in the last instance, where the mosquito after barely succeeding in breaking the skin and inserting its rostrum for 20 seconds, was too exhausted for flight. However, as one may find in the description of the dissected specimen, sporozoites were still present in moderate numbers five hours after the last bite mentioned.

Plate I is a photograph of one of the crushed salivary lobes representing the degree of parasitism found in mosquito No. 23, 25 days after only a single biting of the original tertian donor. It is to be noted that the mosquito had been given an opportunity to exhaust its sporozoites in the process of biting 12 healthy persons during February 17 to March 1. Nine of these volunteers gave ample evidence of having received infective sporozoites, and in three of these infections mosquito No. 23 was wholly responsible.

DISCUSSION OF PRINCIPLES INVOLVED.

The factors governing the power to convey infection are regarded as dependent upon:

(*a*) The number of sporozoites in the salivary glands.

(*b*) The number of sporozoites discharged at each feeding.

(*c*) The viability and longevity of the sporozoites in the glands.

The loss of power to convey infection in this species of *Anopheles* probably depends on the elimination of the sporozoites from the salivary glands through discharge or death of these organisms.

(*a*) The number of sporozoites in the salivary glands would depend on the degree of infection, namely, the number of ripe oocysts and the number of expelled oocyst sporozoites which succeed in migrating into the glands.

(*b*) The number of sporozoites discharged at each feeding would likely depend on the length of time the mouth parts of the mosquito were applied in one or more feedings. This factor would probably be influenced by the salivary discharge, whether continuous or not. If we take for granted that the stomach oocysts have exhausted themselves as to sporozoite production and that gland sporozoites only are to be accounted for, then only the interval during feedings must be taken into consideration; providing also that the salivary glands have attained their full virulence; that is, have become invaded by all of the sporozoites originally discharged into the cœlom or at least until the time when no more sporozoites enter the glands.

The number of sporozoites injected into the intermediate host may depend in a measure on the duration of the bite. It has been demonstrated by Nuttall and Shipley (1901) (Studies in Relation to

Malaria, Jour. of Hygiene, vol. 1) that the quantity of saliva is in direct proportion to the length of time the proboscis of the mosquito is inserted.

Ross (1910, p. 88) has stated in commenting on the numbers of protospores (sporozoites) which enter the human blood: "This must depend (*a*) upon the number of spores in the biting insect's salivary glands, and (*b*) upon the number of times it is allowed to bite its victim. I think that mosquitoes inject their poison before commencing to suck. If this is the case an insect which bites a person several times (as, for instance, when he is asleep) is likely to inoculate many more protospores than one which succeeds in biting only once," (*c*) the viability and longevity of the sporozoites in the glands may possibly be influenced by temperature and diet. Investigations have tended to show that these factors affect the development of the zygote preceding the formation of mature oocysts so that their relation to gland sporozoites need not be considered here.

SUMMARY.

In 17 experiments in which human beings were employed to test the infectibility of *Anopheles punctipennis* with *Plasmodium vivax*, 14 cases of malarial fever resulted. The sporozoites in the mosquitoes used developed 10–22 days after the definitive hosts were given an opportunity to bite a patient harboring a scanty number of mature tertian gametocytes.

In an attempt to infect several persons with a single specimen of *Anopheles punctipennis*, one mosquito proved to be the sole infective agent in one experiment and one proved to be the sole infective agent in three experiments. These two specimens when applied to the same person transmitted the infection in five cases, while one of them used with a third mosquito succeeded in infecting four persons.

In these experimental inoculations it was demonstrated that in nine instances in which two mosquitoes succeeded in transmitting malaria at least one of the pair was proved to be capable of causing the disease when used singly.

It was demonstrated in 11 experiments that short exposure to bites was sufficient to cause successful transmission of the disease.

In all of the successful inoculations only tertian infection was reproduced. *Plasmodium vivax* was demonstrated microscopically.